Plant Physiol. (1978) 62, 243-248

Cyanide-resistant Respiration in Fresh and Aged Sweet Potato Slices[1]

Received for publication August 8, 1977 and in revised form March 23, 1978

ATHANASIOS THEOLOGIS AND GEORGE G. LATIES[2]
Department of Biology and Molecular Biology Institute, University of California, Los Angeles, California 90024

ABSTRACT

The respiration of fresh sweet potato (*Ipomoea batatas*) slices is resistant to, and often stimulated by, cyanide and antimycin A. *m*-Chlorobenzhydroxamic acid (CLAM), a selective inhibitor of the alternate path, inhibits respiration in the presence of cyanide and has a limited inhibitory effect in the presence of antimycin A. Thus, a partial bypass of the antimycin-sensitive site is indicated. Respiration rises 2-fold at best with slice aging, the increment being cytochrome-mediated. The cyanide-resistant pathway contributes neither to coupled fresh slice respiration nor to the induced respiration in the absence of inhibitors of the cytochrome path. In the presence of uncoupler, however, the alternate path is engaged both in fresh and aged slices. V_{cyt}, the maximal capacity of the cytochrome path, remains essentially the same with slice aging, whereas V_{alt} decreases from 20 to 60 per cent. The induced respiration is readily accommodated by the potential cytochrome path capacity of fresh slices, which is realized on aging. Accordingly, there is no need to invoke mitochondrial proliferation in explanation of the development of the induced respiration. The engagement of the alternate path in response to uncoupler reflects substrate mobilization to a degree that substrate oxidation exceeds the electron transport capacity of the cytochrome path.

Fresh slices do not utilize exogenous substrates, whereas aged slices do so readily. Cerulenin, a specific inhibitor of fatty acid synthesis, prevents the development of the induced respiration as well as the capacity to oxidize exogenous substrates. It is suggested that lipid, and ultimately membrane, biosynthesis is central to the development of the induced respiration and the ability to use exogenous substrates, much as in potato.

When bulky plant storage organs are cut into thin slices there is an immediate rise in respiration. In the 24 hr following cutting the respiration rises another 2- to 5-fold, depending on the tissue, to yield the wound-induced, or induced, respiration (8, 24). We have found storage organs to fall into two groups. In one group, of which potato is typical, the respiration of fresh slices is predominantly CN-sensitive, and slicing elicits a spate of phospholipid degradation (20). In the other group, of which sweet potato is an example, fresh slice respiration is largely CN-insensitive, and cutting evokes no perceptible phospholipid breakdown.

When white potato slices are aged, the development of the induced respiration goes hand in hand with the development of CN resistance. Although the capacity of the CN-resistant, or alternate, path is sufficient to accommodate the induced respiration, the respiration of coupled aged potato slices has been found to be mediated entirely by the Cyt path (21). Further, the maximal potential Cyt path activity in potato has been found to be essentially the same in fresh and aged slices, the induced respiration representing the realization of the latent capacity in fresh slices (21). Thus, the imputation of the induced respiration to a proliferation of mitochondria (11, 16) is untenable. Finally, the impairment of fatty acid or phospholipid synthesis during aging prevents the development of the induced respiration as well as CN resistance in potato slices (25, 26). We take this to reflect the central role of membrane biosynthesis in the developmental process.

In this paper we examine the respiration of sweet potato slices, initially CN-resistant, with respect to the extent and nature of the induced respiration. We estimate the maximal capacity of the Cyt and CN-resistant paths in fresh and aged slices, as well as the extent of alternate path contribution to the total respiration in the absence of CN in both coupled and uncoupled slices. Finally, we investigate the question of whether phospholipid biosynthesis remains a requirement for the development of the induced respiration in a tissue which shows no overt lipid breakdown on slicing.

MATERIALS AND METHODS

Plant Material. Red sweet potato roots (*Ipomoea batatas*) were obtained from a local market and stored at 7 C and 90% RH.

Slice Preparation—Respiratory Measurements. Sweet potato slices 1 mm thick and 9 mm in diameter were prepared as previously described (21). Slices were aged by incubating discs in 0.1 mM $CaSO_4$ with frequent changes for 24 hr at 25 C on a rotary shaker. Respiratory rates were determined by conventional manometry. Cerulenin was dissolved in a minimum amount of alcohol (1 mg of cerulenin/75 μl of alcohol) and diluted with water to give a stock solution of 1 mg/ml. Slices were aged in cerulenin at a concentration of 0.1 mg/ml 0.1 mM $CaSO_4$. CCCP was 10 μM in 0.01 M phosphate buffer—0.1 mM $CaSO_4$ (pH 7.3) where indicated.

Analysis of Titration Data. Titration of sweet potato slice respiration with CLAM[3] (17) and CN or antimycin A was conducted by the method of Bahr and Bonner (2). The equation describing the total respiration of sweet potato slices is:

$$V_T = \rho \cdot g(i) + V_{cyt} + V_{res} \quad (1)$$

where V_T is the total respiration rate, V_{cyt} is the contribution by the Cyt path, V_{res} is the rate of the residual respiration which is uninhibited by both KCN and CLAM together, and g(i) is the maximal contribution by the alternate path at given concentrations of alternate path inhibitor, *e.g.* CLAM. ρ, a number between 0 and 1, defines the fraction of the full alternate path which is operating. V_{alt} is the maximal capacity of the alternate path, and $\rho \times V_{alt}$ is the actual contribution of the alternate path in the absence of inhibitor. When V_{res}, which is constant, is subtracted,

[1] This work was supported by United States Public Health Service Grant GM 19807 to G. G. L.

[2] Address reprint requests to Dr. G. G. Laties, Department of Biology, University of California, Los Angeles, Calif. 90024.

[3] Abbreviations: CLAM: *m*-chlorobenzhydroxamic acid; CCCP: carbonyl-cyanide *m*-chlorophenyl hydrazone; cerulenin: (2S) (3R) 2,3 epoxy-4-oxo-7,10 dodecadienoylamide; TCAC: tricarboxylic acid cycle; SHAM: salicylhydroxamic acid.

we have equation 2, in accordance with the expression developed for mitochondrial respiration (2, 21):

$$V_T' = \rho \cdot g(i) + V_{cyt} \quad (2)$$

Oxidation of Exogenous Labeled Substrates. Forty-two slices (about 3 g fresh wt) were placed in 10 or 15 ml of solution in a 125-ml Erlenmeyer flask. Labeled compounds were added as follows: 10 μCi of [1,5-^{14}C]citrate (4 mCi/mmol), or 10 μCi of uniformly ^{14}C-labeled glucose (200 mCi/mmol), or 10 μCi of [1,2-^{14}C]acetate (56.7 mCi/mmol). Samples were incubated in a water bath rotary shaker at 25 C. Respiratory CO_2 was absorbed in 0.2 ml of 10% NaOH dispersed on a strip of Whatman Gf/A glass paper (1 × 8 cm) bent into a loop and suspended from a hook fixed into the center of a rubber stopper tightly held in the top of the Erlenmeyer flask (7). Slices were incubated for 2 hr. The NaOH loops were changed every 20 min. The loops were dried in an oven at 80 C, and then added directly to a vial with 15 ml of toluene containing 4 g of PPO and 0.05 g of POPOP/liter. Radioactivity was determined with a Beckman scintillation counter model LS-100-C. Duplicate samples were counted for 10 min each. $^{14}CO_2$ evolution is expressed as dpm/3 g fresh wt·hr.

Biochemicals. CCCP and antimycin A were obtained from Sigma. UL-[^{14}C]Glucose and [1,2-^{14}C]acetate were from ICN. [1,5-^{14}C]Citrate was purchased from New England Nuclear. CLAM was synthesized as previously described (21). PPO was obtained from Amersham/Searle, and POPOP was purchased from Nuclear-Chicago.

RESULTS

EFFECT OF KCN, ANTIMYCIN A, AND CLAM ON FRESH SLICE RESPIRATION

Figure 1 shows the effect of CN and antimycin A on the respiration of fresh sweet potato slices in the presence and absence of CLAM (17). Conventional concentrations of CN (*viz.* 0.5 mM) or antimycin (10 μM) stimulate the respiration of coupled fresh sweet potato slices 24 and 34%, respectively, whereas the same inhibitor concentrations resulted in 80% inhibition in fresh potato slices (21). At high CN concentration in the presence of 1 mM CLAM, fresh slice respiration is inhibited 86% (Fig. 1A). The residual respiration (14%) is resistant to CN and CLAM together. On the other hand, at high antimycin concentration in the presence of 1 mM CLAM respiration is inhibited only 56% (Fig. 1B). The weak synergistic effect of antimycin and CLAM is reminiscent of that observed in aged potato slices, where in the presence of CLAM a significant fraction of the CN-sensitive respiration is mediated via an antimycin-resistant branch of the Cyt path (22).

CONTRIBUTION OF ALTERNATE PATH IN COUPLED AND UNCOUPLED FRESH SLICES

Values of ρ in Coupled and Uncoupled Fresh Slices as Measured with CN and CLAM. The synergistic effect of KCN and CLAM on fresh slice respiration (Fig. 1A) establishes the presence of the alternate path in fresh sweet potato slices. Titrations of respiration in coupled and uncoupled fresh slices with CLAM in the presence and absence of CN are shown in Figure 2. Figure 2A shows that in coupled slices CLAM at concentrations as high as 4 mM does not inhibit the respiration in the absence of CN. However, in the presence of 0.1 mM KCN, CLAM results in 85% inhibition.

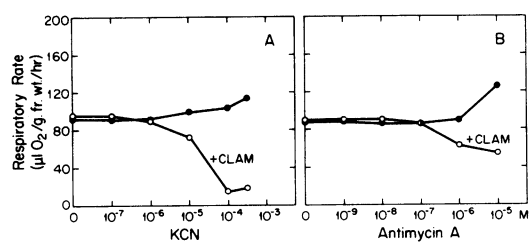

FIG. 1. Effect of CN and antimycin A with and without CLAM on the respiration of coupled fresh sweet potato slices. A: KCN; B: antimycin A. CLAM concentration: 1 mM.

The data in Figure 2A were replotted according to equation 2, and the results are shown in Figure 2B. A horizontal line is obtained, with slope (ρ) of 0, indicating that the alternate path does not contribute to the respiration in the absence of CN. Experiments similar to those shown in Figure 2A were carried out in the presence of CCCP, and the results are presented in Figure 2C. In the absence of CN, CLAM partially inhibits respiration, the respiratory rate reaching a plateau at 4 mM CLAM. In the presence of CLAM and 0.1 mM KCN almost complete inhibition is obtained (Fig. 2C). When titration with CLAM is carried out with low CN (0.01 mM KCN), the inhibitory pattern of CLAM remains the same, although inhibition is less severe.

The data in Figure 2C were replotted according to equation 2 and the results are shown in Figure 2D. In the absence of CN, a straight line is obtained with slope (ρ) of 0.9, indicating 90% engagement of the alternate path. On the other hand, in the presence of 0.01 mM KCN, a straight line with slope (ρ) of 1 is obtained, with its intercept on the y axis lowered, indicating that the alternate path operates at its maximum capacity while the rate of the Cyt path (V_{cyt}) has been reduced.

Quantitative Relations of V_{cyt}, V_{alt}, and V_{res} in Coupled and Uncoupled Fresh Slices Estimated with CN and CLAM. The respiratory fluxes of coupled and uncoupled fresh sweet potato slices determined from Figure 2, B and D, are summarized in Table I. Data from another set of experiments are also presented (Table I, experiments 4–6). The main difference between the two sets of experiments is that in the first set (experiments 1–3) CN stimulates the respiration, and V_{alt} is thus higher than V_{cyt}, whereas in the second set (experiments 4–6) CN slightly inhibits the

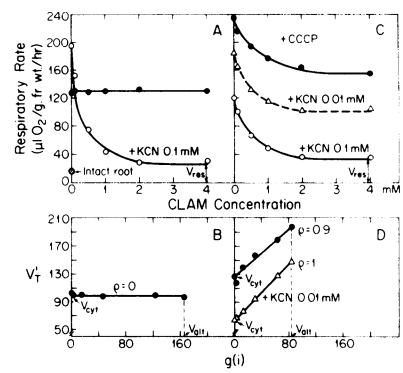

FIG. 2. Effect of CLAM with and without CN on coupled and uncoupled fresh sweet potato slices. A and B: coupled; C and D: uncoupled. g(i) in B and D derived from A and C, respectively. V_{res} is subtracted in calculating g(i). V_T' and g(i) are in μl of O_2/g fresh wt·hr.

Table I. Respiratory Rates of Coupled and Uncoupled Fresh Sweet Potato Slices.

Expts. 1, 2 and 3 were carried out with slices from a single sweet potato purchased in April. The data were obtained from Fig. 2A, B, C, D. Expts. 4, 5 and 6 were performed with slices from a single sweet potato purchased in September.

Experiment	10 µM CCCP	0.01 mM KCN	V_T	V_{alt}	ρ	$\rho \times V_{alt}$	V_{cyt}	V_{res}
			µl O_2/g fresh wt·hr				µl O_2/g fresh wt·hr	
1	-	-	127	165	0	0	97	30
2	+	-	235	83	0.9	75	123	37
3	+	+	185	83	1	83	65	38
4	-	-	110	77	0	0	90	20
5	-	+	105	63	0.5	31.5	56	20
6	+	-	186	61	1	61	105	23

respiration and V_{alt} is accordingly less than V_{cyt}. The above discrepancy depends on the storage history of the root. Freshly dug roots (purchased in September) have a smaller alternate path capacity than do roots stored for long periods (purchased in April). In both cases in coupled slices in the absence of CN (experiments 1 and 4) V_T is the sum of V_{cyt} and V_{res}. The contribution of the alternate path is zero, since ρ is 0. When the activity of the Cyt path of coupled slices is decreased by a low concentration of CN (experiment 5) ρ is shifted to 0.5, and the contribution of the Cyt path decreases, without any change in V_T compared with experiment 4.

Uncouplers of oxidative phosphorylation stimulate fresh sweet potato slice respiration. Accordingly, V_T increases (compare experiment 1 with experiment 2, or experiment 4 with 6) and the alternate path is engaged, since ρ is equal to 1. The activity of the Cyt path increases 16 to 27% with CCCP (compare the V_{cyt} values of experiment 1 and 2 or 4 and 6), indicating that in coupled slices the Cyt path operates below its full potential capacity. Thus, the bulk of the uncoupler-evoked increment is alternate path-mediated. The decrease of V_T in the presence of 0.01 mM KCN in uncoupled slices is due to a decrease in V_{cyt}, the contribution of the alternate path remaining the same ($\rho = 1$).

The engagement of the alternate path in the presence of uncoupler is not always observed. Table II shows the values of ρ, V_{cyt}, V_{alt}, and V_{res} estimated in the presence and absence of CCCP in fresh slices prepared from two different roots purchased in April (experiments 1 and 2) and September (experiments 3 and 4), respectively.

V_T increases 65% in response to uncoupler (compare V_T values of experiment 1 with 2 or experiment 3 with 4), but the alternate path remains disengaged as indicated by the value of ρ equal to 0. Whereas V_{cyt} values increased 16 to 27% in response to CCCP in Table I, V_{cyt} increased 65 to 83% in the experiments of Table II, indicating that the fresh slices described in Table II have a much higher potential V_{cyt} than those in Table I. The above observation implies that since V_{cyt} in Table II is much higher than that in Table I, substrate mobilization caused by uncouplers is not enough to saturate both pathways. The engagement of the alternate path is seemingly regulated by the traffic delivered to the electron transport chain.

Values of ρ, V_{cyt}, V_{alt}, and V_{res} Estimated with Antimycin A and CLAM in Fresh Coupled and Uncoupled Slices. The magnitude of V_{cyt}, V_{alt}, and V_{res} shown in Tables I and II was determined by titrating the alternate path with CLAM in the presence and absence of CN, an inhibitor of Cyt oxidase. Since the alternate path branches from the Cyt path in the ubiquinone region before Cyt *b* (19), antimycin A, a specific inhibitor of complex III (15), should substitute for CN in titrations with CLAM.

The results of titrating coupled and uncoupled fresh sweet potato slices with CLAM in the presence and absence of 10 μM antimycin are shown in Figure 3. CLAM at a concentration as high as 4 mM does not inhibit the respiration in the absence of antimycin. Respiration is partially sensitive to CLAM in the presence of antimycin as shown in Figure 3A. CLAM in the presence of 10 μM antimycin results in 60% inhibition, compared with 85% inhibition in the presence of 0.1 mM KCN (Fig. 2A). In Figure 3B, V_T' is plotted as a function of g(i), the data deriving from Figure 3A. A straight line with slope $\rho = 0$ is obtained, indicating that the alternate path does not contribute to V_T'.

Similar titration in the presence of CCCP is shown in Figure 3C. In the absence of antimycin, CLAM partially inhibits respiration, the respiratory rate reaching a plateau at high CLAM concentrations. In the presence of antimycin inhibition is less than with CN, and V_{res} is accordingly unduly high. In Figure 3D, V_T' is plotted against g(i) with data from Figure 3C. A straight line with slope $\rho = 0.8$ is obtained, indicating 80% engagement of the alternate path.

The respiratory fluxes of coupled and uncoupled fresh sweet potato slices determined from Figure 3, A, B, C, and D, are summarized in Table III. The weak synergistic effect of antimycin and CLAM results in an overestimation of V_{res} (compare V_{res} values of experiment 1 in Table III with those of experiment 1 in Table I), with the result that V_{cyt} and V_{alt} are underestimated (compared V_{cyt} and V_{alt} values of experiment 1 in Table III with those of experiment 1 in Table I). Antimycin is seen to be an unsuitable inhibitor in studies designed to determine the magnitude of the Cyt and alternate paths in fresh sweet potato slices, since it fails to inhibit the Cyt path completely. Antimycin is even less effective in aged slices (see Table V).

Effect of Uncouplers on Activity of Alternate Path. Figure 4 shows g(i) values in the presence of uncoupler plotted against g(i) values in the absence of uncoupler with g(i) values determined with KCN (plot A) and antimycin (plot B), respectively. In both instances straight lines are obtained, but the slope derived with KCN is 0.6, whereas the slope derived with antimycin is 0.9 to 1. A slope less than 1 indicates that the activity of the alternate path has been decreased by uncoupler.

Although the effect of uncoupler on g(i) as determined with CN suggests that the energy state regulates the activity of the alternate path, the absence of a similar effect of uncoupler on g(i) as

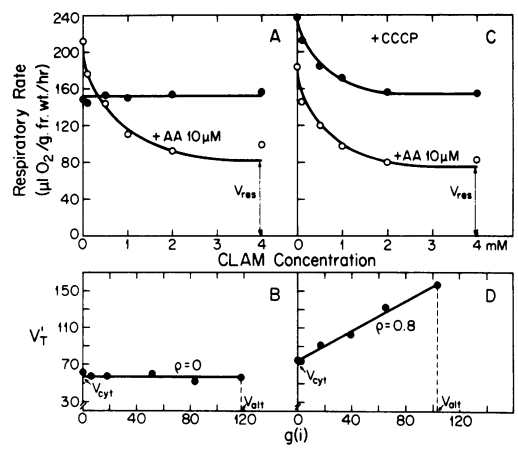

FIG. 3. Effect of CLAM in presence and absence of antimycin A on coupled and uncoupled fresh sweet potato slices. A and B: coupled; C and D: uncoupled. g(i) in B and D derived from A and C, respectively. V_{res} is subtracted in calculating g(i). V_T' and g(i) are in μl of O_2/g fresh wt·hr.

Table II. Respiratory Rates of Coupled and Uncoupled Fresh Sweet Potato Slices.

Data were obtained from analyses similar to those of Figure 2. Expts. 1 and 2 were from the same root purchased in April. Expts. 3 and 4 were from the same root purchased in September.

Experiment	10 µM CCCP	V_T	V_{alt}	ρ	V_{cyt}	V_{res}
		µl O_2/g fresh wt·hr			µl O_2/g fresh wt·hr	
1	-	135	149	0	107	28
2	+	224	96	0	196	25
3	-	94	90	0	80	14
4	+	156	35	0	133	23

Table III. Respiratory Rates of Coupled and Uncoupled Fresh Sweet Potato Slices.

The data were obtained from Figure 3A, B, and C. Expts. 1 and 2 were from the same root.

Experiment	10 µM CCCP	V_T	V_{alt}	ρ	$\rho \times V_{alt}$	V_{cyt}	V_{res}
		µl O_2/g fresh wt·hr				µl O_2/g fresh wt·hr	
1	-	149	119	0	0	56	93
2	+	238	103	0.8	82	74	81

determined with antimycin argues against this view. Seemingly, CN decreases V_{alt} in uncoupled slices in a nonspecific way. The underestimation of g(i) in uncoupled slices resulting from the nonspecific effect of CN leads to an overestimation of ρ, which has been found to be 0.9 to 1 in uncoupled slices. The true value of ρ in this case is about 0.5 to 0.6. Furthermore, the value $\rho = 0.8$ obtained with antimycin in uncoupled slices (Table III) is also overestimated, since the unduly high values of V_{res} result in an underestimation of g(i). A ρ equal to 0.5 is closer to reality.

Inhibitor Constant of CLAM. The inhibitor constant (K_i) was determined from Dixon plots of the reciprocal of the respiratory rate in the presence of 0.1 mM CN, against CLAM concentration (4). Table IV compares K_i values of CLAM in fresh sweet potato slices with those reported in other tissue slices. The values are half those reported for *Arum* and avocado slices, and are very similar to those reported for aged potato and fresh preclimacteric banana slices. The similarity in K_i values suggests that the hydroxamate-sensitive component may well be of a similar nature among the various tissues, with the possible exception of *Philodendron* spadix slices.

Quantitative Relations of V_{cyt}, V_{alt}, and V_{res} in Fresh and Aged Slices. The respiratory fluxes of coupled and uncoupled fresh and aged sweet potato slices determined from titrations with CLAM in the presence of CN are summarized in Table V. Slicing an intact sweet potato root results in an 8- to 10-fold increase in respiration. The wound respiration is the sum of V_{cyt} and V_{res}, the contribution of the alternate path to the fresh slice respiration being zero ($\rho = 0$; Table V, experiment 1). Uncouplers stimulate fresh slice respiration and partially engage the alternate path ($\rho = 0.25$; Table V, experiment 2). Thus, the bulk of the uncoupled respiratory rate (90%) is Cyt path-mediated. The above observation indicates an excess of unexpressed Cyt path capacity in fresh slices (compare the V_{cyt} values of experiment 1 with experiment 2).

The induced respiration in aged sweet potato slices represents a modest 30% increase over the fresh slice rate as contrasted with white potato slices where aging results in a 4- to 5-fold increase of respiration (5, 21). The maximum increase in V_T during aging of sweet potato slices has never been observed to be more than 2-fold. Much as in white potato, however, the maximal potential capacity of the Cyt path, *i.e.* that realized in the presence of uncoupler, is much the same in fresh and aged slices. In sweet potato slices, however, V_{alt}, the maximal capacity of the alternate path, *decreases* with aging, while the actual Cyt path contribution in coupled slices increases (Table VA). By contrast, the alternate path is absent in fresh white potato slices and well developed in aged. In Table VB, we see that antimycin inhibition in aged slices is incomplete (22), with the result that V_{cyt} is grossly underestimated and V_{res} is grossly overestimated.

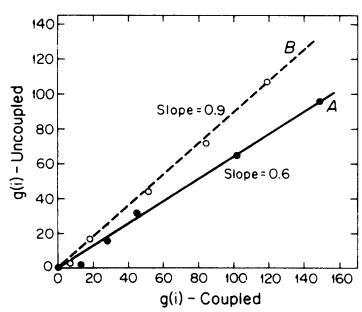

FIG. 4. Effect of uncouplers in fresh sweet potato slices on function, g(i), as measured in presence of CN (A) or antimycin A (B).

Table IV. Inhibitor Constant (K_i) for CLAM in Coupled Fresh Sweet Potato Slices and in Other Tissues.

Value for fresh Arum spadix from Wedding et al. (27). All others determined by authors.

Tissue slice	K_i
	μM
Fresh sweet potato	190-240
Aged white potato	240-310
Fresh preclimacteric banana	120-170
Fresh preclimacteric avocado	600
Fresh Philodendron spadix	100
Fresh Arum spadix	490

Table V. Respiratory Rates of Coupled and Uncoupled Fresh and Aged Sweet Potato Slices.

All experiments were from the same root. Aged slices, 24 hr. KCN, 0.1 mM, antimycin 10 μM, CLAM, 1 mM.

A. Values determined with KCN and CLAM

Tissue	Experiment	CCCP 10 μM	V_T	V_{alt}	ρ	$\rho \times V_{alt}$	V_{cyt}	V_{res}
			μl O_2/g fresh wt·hr				μl O_2/g fresh wt·hr	
Fresh	1	-	105	89	0	0	54	51
	2	+	186	74	0.25	18	126	43
Aged	3	-	136	35	0	0	96	40
	4	+	196	38	1	38	100	59

B. Values determined with antimycin and CLAM

Fresh	5	-	105	50	0	0	29	75
Aged	6	-	136	17	0	0	9	131

The data of Table V indicate that substrate mobilization caused by uncouplers in fresh slices is not enough to saturate both the Cyt and alternate paths, with the result that $\rho = 0.25$. On the other hand, in aged slices substrate mobilization by uncoupler saturates both pathways despite the quantitative similarity between uncoupled V_T in fresh and aged slices. The above discrepancy is attributable to the reduction of V_{alt} in aged slices.

In sum, the potential Cyt path capacity of fresh sweet potato slices is enough to sustain the respiration of aged slices, and the CN-resistant pathway does not contribute to the development of the induced respiration. In consequence, there is no need to invoke the proliferation of mitochondria in explanation of the development of the induced respiration in sweet potato slices (1, 16).

Utilization of Exogenous Substrates by Fresh and Aged Slices. Table VI shows the $^{14}CO_2$ evolution from uniformly labeled [^{14}C]glucose, [1,5-^{14}C]citrate, and [1,2-^{14}C]acetate by fresh and aged sweet potato slices. Aged slices readily oxidize each of the substrates to yield copious quantities of $^{14}CO_2$, whereas fresh slices fail to release significant quantities of radioactive CO_2 from any of the substrates in question. Although glucose, citrate, and acetate uptake increase with aging, absorption by fresh tissue is nevertheless significant, especially where glucose and acetate are concerned. It is evident from Table VI that the enhancement of utilization with aging far exceeds the augmentation of absorption capacity. Further, a restraint on glycolytic and TCAC activity is seemingly lifted with aging in sweet potato slices much as in white potato slices (7) albeit there is no measurable lipid breakdown on slicing in sweet potato (Theologis and Laties, unpublished). Fatty acids arising from lipid breakdown have been considered as the inhibitors of glycolysis and TCAC activity in fresh potato slices (6, 9).

Effect of Cerulenin on Development of Induced Respiration and Glucose Utilization Capacity by Sweet Potato Slices. Cerulenin, an antibiotic which curtails *de novo* fatty acid synthesis by irreversible covalent attachment to β-ketoacyl-acyl carrier protein synthetase (14), inhibits the development of the induced respiration. Table VII shows the effect of cerulenin both on the development of the induced respiration and on the capacity of aged sweet potato slices to utilize exogenous glucose. The respiration of cerulenin-aged slices remains at levels only slightly higher than those observed for freshly cut material. Furthermore, V_{cyt} does not rise in slices aged in cerulenin. Cerulenin inhibits the enhanced utilization of exogenous glucose as well as the increase in glucose absorption by aged slices (Table VIIB).

Table VI. Oxidation of Exogenous Labeled Substrates by Fresh and Aged Sweet Potato Slices.

The radioactivity in 15 ml experimental solution was glucose, (UL ^{14}C) 18.5×10^6 dpm; citrate (1,5 ^{14}C), 20×10^6 dpm and acetate (1,2 ^{14}C) 20.2×10^6 dpm respectively.

Tissue	$^{14}CO_2$ Release			Uptake		
	Glucose	Citrate	Acetate	Glucose	Citrate	Acetate
	dpm $\times 10^{-4}$/3 g fresh wt·2hr			Percent initial radioactivity taken up		
Fresh	0.84	< 1	3.4	9	2.5	21
Aged	165	120	136	96	40	80
Ratio Aged/Fresh	196	> 120	40	11	16	3.8

Table VII. Effect of Cerulenin on the Development of the Induced Respiration and on glucose oxidation in sweet potato slices.

Slices were aged 24 hr where indicated. Initial ($U^{14}C$) glucose radioactivity in 10 ml experimental solution was 18.3×10^6 dpm.

A. Respiration

Treatment	Fresh	Aged	
		Control	Cerulenin
		µl O_2/g fresh wt·hr	
Control	99	192	114
KCN 0.1 mM	152	154	154
CLAM 1 mM	98	186	109
KCN + CLAM	39	55	60
CCCP, 10 µM	182	232	178

Tissue	V_T	V_{alt}	V_{cyt}	V_{res}	V_T uncoupled
	µl O_2/g fresh wt·hr				
Fresh	99	113	59	39	182
Aged	190	99	131	55	232
Aged + Cerulenin	114	94	49	60	178

B. Glucose oxidation

Tissue	$^{14}CO_2$ Release		Uptake	
	dpm $\times 10^{-4}$/g fresh wt·2 hr	Relative rate	Percent initial radioactivity	Relative rate
Fresh	1.2	1	13	1
Aged	184	153	80	6.1
Aged + Cerulenin	1.6	1.3	9	0.7

The above data indicate that fatty acid biosynthesis—and by extension the biosynthesis of membrane-lipid components—is a requirement for the development of the induced respiration and the enhancement of glucose utilization which accompanies aging of sweet potato slices.

DISCUSSION

Cyanide Resistance in Fresh and Aged Slices. It has been axiomatic through the years that the respiration of fresh slices from various storage organs is CN-sensitive and that the development of respiration with slice aging is concurrent with the appearance of the CN-resistant pathway (9, 24). The behavior of potato slices is largely responsible for the erroneous generalization. An extensive comparative study of 20 storage tissues has shown that fresh slices fall into two main categories. The first group includes tissue slices which are initially CN-sensitive and which develop CN resistance with aging. In the second group, fresh slices are resistant and often stimulated by CN, and aging results in some diminution of alternate path activity (Theologis and Laties, unpublished). In both groups slice aging results in the development of a wound-induced respiration, indicating that the enhancement of respiration with aging is independent of whether the alternate path exists in fresh slices.

An extensive study of CN-resistant respiration in potato slices (21), a member of the first group, showed that the alternate path does not normally contribute to the induced respiration in potato slices. In the present study, red sweet potato slices, which belong to the second group, were investigated to determine the extent to which the Cyt and CN-resistant paths contribute to the fresh and induced respiration. As demonstrated, the alternate path exists in both fresh and aged sweet potato slices. However, the respiratory rate of coupled fresh and aged slices, corrected for the residual respiration, has been found to be less than predicted from the sum of the CN-resistant (V_{alt}) and CLAM-resistant (V_{cyt}) rates measured independently, and we have determined that in neither case does the alternate path contribute to the respiration in the absence of inhibitors.

The extent of saturation of the Cyt path determines the flux through the alternate path. When the flux through the Cyt path is decreased by low CN concentrations, the value of ρ shifts from 0 to a value greater than 0. Restriction of Cyt path activity leads to the engagement of the alternate path.

In uncoupled fresh sweet potato slices the alternate path seems fully engaged. That is, the uncoupled respiratory rate corrected for the residual respiration equals the sum of $V_{alt} + V_{cyt}$. The bulk of the respiratory increment induced by CCCP is mediated by the alternate path. However, the somewhat depressing effect of CN on the alternate path in uncoupled slices (Fig. 4) leads to an underestimation of g(i) with a corresponding overestimation of ρ. Thus, the actual participation of the alternate path ranges between 50 and 60% of its maximum capacity when the underestimation of g(i) is taken into consideration. The engagement of the alternate path by uncouplers in aged white potato slices has been attributed to an increase in substrate mobilization due to the enhancement of glycolysis (21). We interpret the response to uncouplers in sweet potato slices in the same way. When substrate oxidation exceeds the electron transport capacity of the Cyt path, the alternate path is engaged. The alternate path is not invariably operative in the presence of uncoupler. When the potential capacity of the Cyt path is large, uncouplers do not cause the engagement of the CN-resistant path (Table II). On the other hand, when the capacity of the alternate path is higher than V_{cyt}, CN will stimulate respiration in the face of a significant Pasteur effect.

Our presumption that substrate flux controls the operation of the alternate path is strengthened by the observation that in mitochondrial studies neither CN nor uncoupler (in state 3) leads to respiratory stimulation, since substrate is normally saturating. In sweet potato mitochondria the value of ρ in state 3 with succinate or malate as substrate is always greater than zero (Grover and Laties, unpublished), whereas in sweet potato slices ρ is equal to 0. Furthermore, whereas in mitochondrial studies the Cyt path is fully saturated (2, 3), in coupled tissue slices the potential capacity of the Cyt path is not always fully utilized (Tables I and V). In practice, the switching mechanism of Bahr and Bonner (3) acts more like an on-off switch than an apportionment regulator. That is, the capacity of the Cyt path must seemingly be exceeded for the alternate path to come into play. The stimulation of respiration by CN in plant tissues has been ascribed to the activation of the alternate path by CN (18). Our results do not support this proposal since antimycin stimulates respiration much as does CN (Figs. 1 and 3).

The weak synergistic inhibitory effect of antimycin and CLAM in fresh slices is reminiscent of that observed in aged potato slices (22). The ineffectiveness of antimycin in inhibiting the Cyt path completely is attributable neither to the impenetrability of the inhibitor nor to the impairment of CLAM effectiveness by antimycin. The data suggest an operational bypass around the antimycin-sensitive site similar to that found in aged potato slices (22). The mitochondrial seat of the antimycin-resistant bypass in sweet potato tissue is implied by the observation of Tomlinson and Moreland (23) that the respiration in the presence of HOQNO plus SHAM in sweet potato mitochondria is higher than that in the presence of KCN plus SHAM, much as in the case of sweet potato tissue slices.

Development of Induced Respiration. The wound respiration of fresh sweet potato slices is 7-fold higher than that of the intact organ (Fig. 2). The fresh slice respiration is resistant to, and often

stimulated by, CN (Fig. 2), contrary to that of the fresh potato slice which is CN-sensitive (21). The synergistic effect of CN and CLAM indicates the presence of the alternate path in fresh sweet potato slices, whereas the alternate path in fresh potato tissue is lacking or inactive (21). The loss of the alternate path in potato slices has been attributed to the extensive membrane-lipid degradation initiated by cutting (20). Consistent with this supposition membrane destruction is not observable in fresh sweet potato slices, where the alternate path can be demonstrated.

The respiration of aged sweet potato slices is at best twice that of fresh slices (Tables V and VII). The alternate path, present in fresh slices, persists at a level which is reduced or at most remains the same (Tables V and VII). Thus, the development of the alternate path, a characteristic of white potato slice aging, is not central to the development of the induced respiration in sweet potato slices. Rather, the induced respiration reflects the realization of the latent Cyt path capacity of fresh slices. Since the alternate path does not normally contribute to the respiration of aged white potato slices, however (21), the difference is not as great as it appears. Whereas fresh slice respiration is stimulated by CN in sweet potato, aged slice respiration is partially inhibited, reflecting the drop in V_{alt} with aging.

When the similar values of V_{cyt} in uncoupled fresh and aged sweet potato slices are taken into account, the premise that mitochondria biogenesis and *de novo* synthesis of respiratory enzymes are necessary for the increase of respiration in sweet potato slices during aging becomes untenable (1, 16). We have come to the view that the potential Cyt path capacity in fresh sweet potato slices is more than enough to sustain the elevated rates of the induced respiration. Aging simply involves the realization of preexisting mitochondrial respiratory capacity. Whereas increased mitochondrial activity may be due to an intrinsic change in the mitochondria (13, 25, 26), the prospect that respiratory enhancement *in vivo* is related to substrate mobilization warrants particular attention.

The inability of fresh sweet potato slices to utilize exogenous substrates (Tables VI and VII) and the dramatic enhancement of exogenous substrate utilization with slice aging recall the situation in white potato slices (8). The behavior of freshly cut potato slices has been attributed to the inhibition of glycolysis and the TCAC by free fatty acids released during cutting (20), and to the prevalence of anomalous α oxidation of fatty acids (6, 10).

The slicing of sweet potato roots does not elicit demonstrable membrane lipid breakdown (Theologis and Laties, unpublished). Furthermore, in contrast to potato, the respiratory increment elicited by uncoupler in fresh sweet potato slices is unaffected by imidazole, a specific inhibitor of fatty acid α oxidation (12, and Theologis and Laties, unpublished). Thus, the explanation adduced heretofore for fresh slice behavior in potato, and its alteration with aging, cannot simply be applied to sweet potato slices. Undoubtedly more is at issue in both cases than has yet been recognized. Nevertheless, the gross transformation of both CN-sensitive and CN-resistant slices with aging must have elements in common, as suggested by the rise in respiration, the burgeoning ability to oxidize substrates, and the repression of the developmental changes in both cases by cerulenin.

Cerulenin, an antibiotic which inhibits *de novo* fatty acid synthesis by irreversible covalent binding to β-ketoacyl-acyl carrier protein synthetase, has been shown to be an effective inhibitor of fatty acid synthesis in yeast, bacteria, and animals (14). Recently it has been shown that cerulenin inhibits the development of the wound-induced and CN-resistant respiration in potato slices, indicating the crucial role of lipid biosynthesis for respiratory enhancement during potato slice aging (26).

The respiration of cerulenin-aged sweet potato slices is much the same as that of fresh discs (Table VII). Cerulenin not only suppresses the development of the induced respiration but also inhibits the uptake and utilization of exogenous substrate. The effect of cerulenin strongly suggests that fatty acid and presumably phospholipid biosynthesis is a crucial element in the development of the wound-induced respiration and substrate transporting systems in sweet potato slices. The nature of the membrane components synthesized during aging, necessary for the realization of preexisting mitochondrial respiratory capacity as well as for the implementation of substrate absorption, remains to be determined.

Acknowledgments—A. Theologis is indebted to UCLA for a predoctoral fellowship and to Phi Beta Kappa for a foreign student predoctoral scholarship. The authors thank S. Ōmura of the Kitasato Institute, School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan, for the kind gift of cerulenin.

LITERATURE CITED

1. ASAHI T, Y HONDA, I URITANI 1966 Increase of mitochondrial content in sweet potato root tissue after wounding. Arch Biochem Biophys 113: 498–500
2. BAHR JT, WD BONNER JR 1973 Cyanide-insensitive respiration. I. The steady states of skunk cabbage spadix and bean hypocotyl mitochondria. J Biol Chem 248: 3441–3445
3. BAHR JT, WD BONNER JR 1973 Cyanide-insensitive respiration. II. Control of the alternate pathway. J Biol Chem 248: 3446–3450
4. DIXON M 1953 The determination of enzyme inhibitor constants. Biochem J 55: 170–171
5. HACKETT DP, DW HAAS, SK GRIFFITHS, DJ NIEDERPRUEM 1960 Studies on the development of cyanide-resistant respiration in potato tuber slices. Plant Physiol 35: 8–19
6. JACOBSON BS, BN SMITH, S EPSTEIN, GG LATIES 1970 The prevalence of carbon-13 in respiratory carbon dioxide as an indicator of the type of endogenous substrate. J Gen Physiol 55: 1–17
7. LATIES GG 1964 The onset of tricarboxylic acid cycle activity with aging in potato slices. Plant Physiol 39: 654–663
8. LATIES GG 1975 Solute transport in relation to metabolism and membrane permeability in plant tissues. *In* PJ Davies, ed. Historical and Current Aspects of Plant Physiology. NY State College of Agriculture and Life Sciences, Cornell University, Ithaca NY, pp 98–151
9. LATIES GG 1978 The development and control of respiratory pathways in slices of plant storage organs. *In* G Kahl, ed. Biochemistry of Wounded Plant Storage Tissues. Walter De Gruyter Publ, Berlin
10. LATIES GG, C HOELLE, BS JACOBSON 1972 α Oxidation of endogenous fatty acids in fresh potato slices. Phytochemistry 11: 3403–3411
11. LEE SG, RM CHASSON 1966 Aging and mitochondrial development in potato tuber tissue. Physiol Plant 19: 199–206
12. MARTIN RO, PK STUMPF 1959 Fat metabolism in higher plants. XII. α Oxidation of long chain fatty acids. J Biol Chem 234: 2548–2554
13. NAKAMURA K, T ASAHI 1976 Changes in properties of the inner mitochondrial membrane during mitochondrial biogenesis in aging sweet potato tissue slices in relation to the development of cyanide-insensitive respiration. Arch Biochem Biophys 174: 393–401
14. ŌMURA S 1976 The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. Bacteriol Rev 40: 681–697
15. RIESKE JS 1976 Composition, structure and function of complex III of the respiratory chain. Biochim Biophys Acta 456: 195–247
16. SAKANO K, T ASAHI 1969 Activation of protein synthesis and biogenesis of mitochondrial particles during aging of disks of sweet potato root tissue. Agric Biol Chem 33: 1433–1439
17. SCHONBAUM GR, WD BONNER JR, BT STOREY, JT BAHR 1971 Specific inhibition of the cyanide-insensitive respiratory pathway in plant mitochondria by hydroxamic acids. Plant Physiol 47: 124–128
18. SOLOMOS T, GG LATIES 1976 Induction by ethylene of cyanide-resistant respiration. Biochem Biophys Res Commun 70: 663–671
19. STOREY BT 1976 Respiratory chain of plant mitochondria. XVIII. Point of interaction of the alternate oxidase with the respiratory chain. Plant Physiol 58: 521–525
20. THEOLOGIS A, GG LATIES 1976 Membrane lipid integrity as a prerequisite element of cyanide-resistant respiration in potato slices. Plant Physiol 57: S-93
21. THEOLOGIS A, GG LATIES 1978 Relative contribution of cytochrome-mediated and cyanide-resistant electron transport in fresh and aged potato slices. Plant Physiol 62: 232–237
22. THEOLOGIS A, GG LATIES 1978 Antimycin-insensitive cytochrome-mediated respiration in fresh and aged potato slices. Plant Physiol 62: 238–242
23. TOMLINSON PF JR, DE MORELAND 1975 Cyanide-resistant respiration of sweet potato mitochondria. Plant Physiol 55: 365–369
24. VAN STEVENINCK RFM 1975 The "washing" or "aging" phenomenon in plant tissues. Annu Rev Plant Physiol 26: 237–258
25. WARING AJ, GG LATIES 1977 Dependence of the wound-induced respiration in potato slices on the time-restricted actinomycin sensitive biosynthesis of phospholipid. Plant Physiol 60: 5–10
26. WARING AJ, GG LATIES 1977 Inhibition of the development of the induced respiration and cyanide-insensitive respiration in potato tuber slices by cerulenin and dimethylaminoethanol. Plant Physiol 60: 11–16
27. WEDDING RT, CC MCCREADY, JL HARLEY 1973 Cyanide sensitivity of respiration during aging of *Arum* spadix slices. New Phytol 72: 15–26